INTRODUCTION

Background

Zinc (Zn) is required micronutrient for organisms in natural waters, functioning as a critical component of various enzyme systems. It occurs at low concentrations in most rivers and estuaries and originates from a variety of natural and anthropogenic sources. Anthropogenic sources of zinc are primarily from industrial and commercial applications, including paint, rubber, dye, wood preservatives, ointments, fertilizers, animal feed, anti-fouling for ships and metal galvanization (Gupta and Karuppiah, 1996; Comber et al. 2002; Heijerick et al., 2002). Contamination from these sources can significantly increase Zn concentrations in impacted riverine and estuarine systems, leading to potential toxicity to organisms.

Dissolved Zn in natural waters may exist as free hydrated ions and as complexes with inorganic and organic ligands (Donat and Bruland, 1995). Some studies suggest that the bioavailability and toxicity of Zn depend upon the activity of the free hydrated ionic form, Zn^{2+}, not simply the total dissolved Zn concentration (Brand et al., 1983; Sunda et al., 1976). In most natural waters, organic ligands exist which can form relatively strong complexes with Zn. Complexation by these organic ligands appears to play a significant role in regulating the biogeochemistry of Zn in natural waters by controlling concentrations of the free hydrated ion. Complexation may also affect the transport and fate of Zn by influencing particle-solute interactions, particularly in estuarine waters containing relatively high levels of particulate material and changing salinities (van den Berg et al., 1987).

Previous studies have detected one or two classes of Zn complexing ligands in estuarine waters. These ligand classes have conditional stability constants (K^{cond}) ranging from $10^{7.4}$ to $10^{9.4}$ and concentrations of 4-160 nM (van den Berg and Dharmvanij, 1984; van den Berg et al., 1986, 1987; Muller and Kester, 1991; Gardner, 1999). Percentages of organically complexed Zn in estuaries range widely from 1 to >95%, depending on the ligand strength, ligand concentration, and the concentration of total dissolved Zn (TDZn). In certain polluted estuaries, total Zn concentrations may saturate the available organic Zn-complexing ligands, leaving an appreciable fraction of Zn in the inorganic form (van den Berg and Dharmvanij, 1984), which may increase its bioavailability and thus increase toxicity to certain organisms (Sunda et al., 1990). However, in evaluating the bioavailability of Zn species, Bruland (1989) suggested that organically complexed Zn might be utilizable by phytoplankton. The nature of Zn complexing ligands is not known; therefore it is difficult to evaluate the exact factors that control Zn toxicity and bioavailability.

The Cape Fear River in southeastern North Carolina has recently been the focus of concurrent studies of benthic fluxes and speciation of dissolved Zn and copper (Cu). This provides a unique opportunity to compare changes in speciation and sediment water interactions for both Zn and Cu. In previous studies, O'Connell (1999) found total dissolved Zn concentrations throughout the Cape Fear estuary (CFE) ranged from 15-90 nM. In the lower CFE, MacGillivray (2002) found total dissolved Zn concentrations ranging from 5 to 10 nM and Zn ligand concentrations ranging from 36 to 96 nM. The excess Zn-complexing ligand relative to TDZn in the CFE reported by MacGillivray, suggests the importance of organic complexation of Zn in the CFE.

The Role of Sediments

Most previous work on Zn speciation in estuaries has focused solely on the concentrations of complexing ligands in the water. However, sediments may also be an important source of ligands to the overlying water column. Preliminary studies by van den Berg and Dharmvanij (1984) suggest sediment porewaters are enriched in Zn-complexing ligands relative to overlying waters. Their results show porewaters contain 16 to 2,000 nM of Zn-complexing ligands with values of K^{cond} of $10^{7.6}$ to $10^{9.3}$. These high ligand concentrations caused a large fraction (typically >90%) of TDZn in porewaters to exist as organic complexes. In general, the porewater ligand concentrations were much greater than overlying water concentrations, suggesting the possibility of a flux of these ligands from estuarine sediments. Byers (1999) determined Zn-ligand fluxes ranging from 144 to 1349 nmol m^{-2} d^{-1} out of the sediment using porewater profiles of Zn ligands in the Elizabeth River, Virginia. At one station in the lower Cape Fear estuary, MacGillivray (2002) measured Zn ligand fluxes directly using sediment core incubations; these fluxes ranged from -980 to 1200 nmol m^{-2} d^{-1}, where positive fluxes are out of the sediment and negative fluxes are into the sediment.

The limited data on metal speciation in estuarine porewaters demonstrate that sediments may be a source of the Zn ligands which regulate Zn speciation, and by extension, Zn bioavailability and potential toxicity, in shallow water environments such as estuaries. This work focuses on the contribution of dissolved Zn and Zn ligands from benthic fluxes over a 2 ½ year period at two sites in the CFE. During this time there were simultaneous measurements of Cu speciation by Shank (2003), providing a comparison of Zn and Cu speciation and sediment-water interactions. In addition, this study provides

the first insights into the chemical nature of Zn-complexing ligands by examining the role of CFE humics in Zn speciation. Information regarding fluxes of metal complexing ligands in estuarine environments is critical for understanding how sediment-water exchange affects the speciation and potential bioavailability of these metals to overlying estuarine waters.

EXPERIMENTAL

Study Location

The Cape Fear River is the largest river basin in the state of North Carolina. In the estuarine portion of the river near Wilmington, NC, two coastal plain rivers, the Black and Northeast Cape Fear rivers, contribute organic-rich freshwater to the mainstem river. Seawater sources include the Atlantic Ocean and the Atlantic Intracoastal Waterway at the seaward end of the estuary. In general, the estuary is slightly stratified because of the relatively short water residence time and rapid flow. The Cape Fear estuary hosts two important shipping terminals: the Military Ocean Terminal Sunny Point (MOTSU) near the seaward end and the Port of Wilmington near the landward end. The Cape Fear River is also a sink for both treated and untreated wastewaters. Discharges into the river basin consist of point sources such as shipping, industry, and municipal wastewater, as well as non-point sources including runoff from agricultural operations and developed lands (e.g. paved surfaces, golf courses, and residential areas).

Sampling

Sediment and water sampling were conducted at two sites in the CFE, each of which has different water chemistry regimes and sediment characteristics (Figure 1). Station 1 was located in the middle estuary where salinities typically range from 10 to 20.

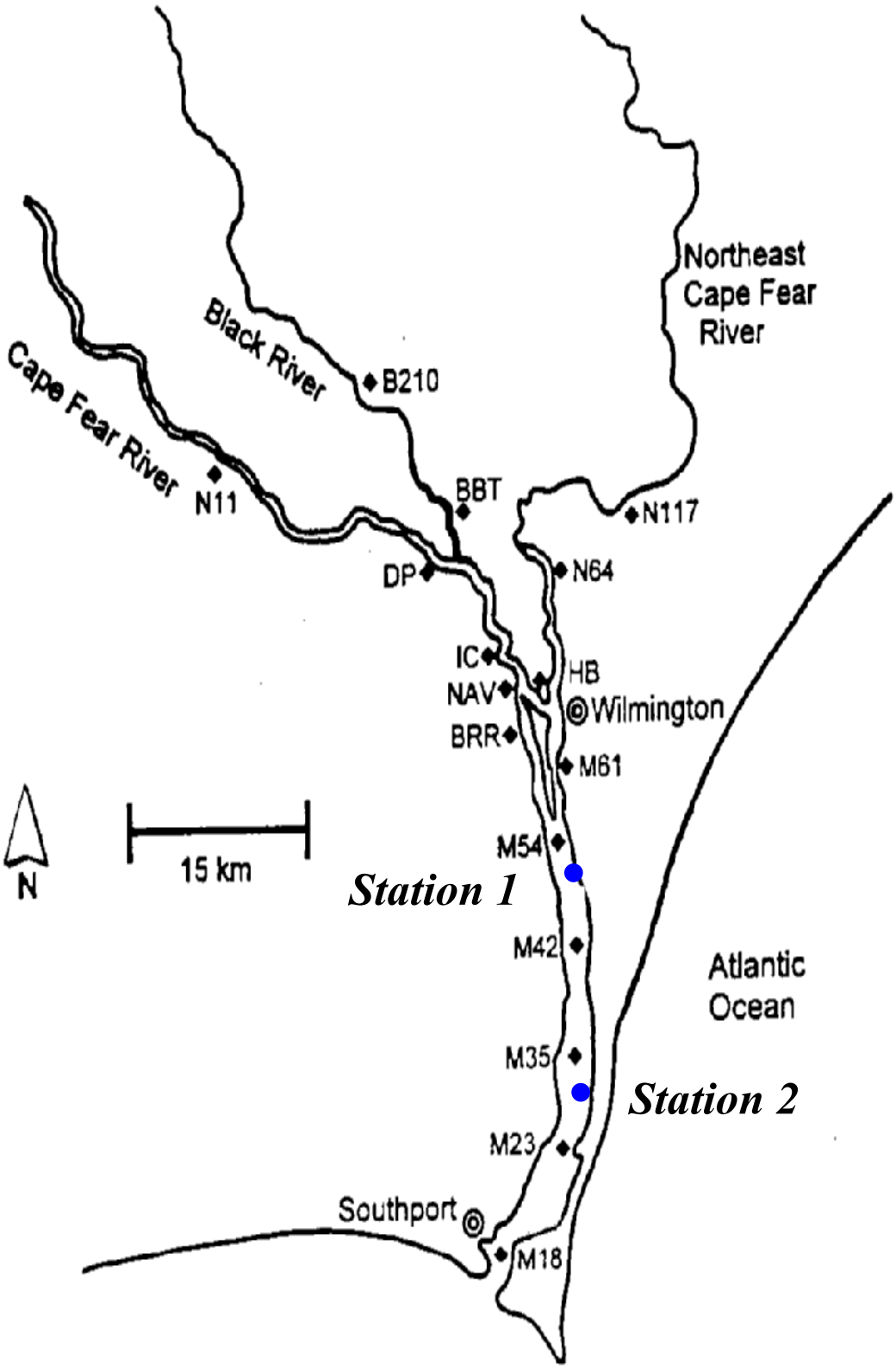


Figure 1. Map of the Cape Fear estuary showing the two sampling sites, Station 1 and Station 2.

Sediments collected at this site were mostly sandy muds with some sulfide present during summer months. Station 2 is a high salinity site (usually > 20) located in the lower Cape Fear. Sediments from this site were predominately muddy sands that also had some sulfide present during the summer months.

Seasonal variability in sediment-water exchange of Zn and Zn-complexing ligands at each of these sites were determined by measuring benthic fluxes during the spring, summer, fall, and winter-spring. Sediments and surface water samples were collected at six different time periods: November 2000, April 2001, June 2001, October 2001, March 2002 and June 2002.

Benthic fluxes of TDZn and dissolved Zn-complexing ligands were measured using a core incubation technique described by Burdige and Homstead (1994), modified for trace metals as described in Skrabal et al. (1997). In this approach, a box corer deployed off UNCW's 19 m research vessel, the R/V *Cape Fear*, collected undisturbed bottom sediments in the estuary. The box cores were carefully subcored using acrylic core tubes (~60 cm long , ~14 cm diameter). Three to six cores were taken at each site with approximately 25 cm of sediment collected in each core. Cores were sealed at the bottom with plastic core caps and covered with acrylic cover plates fitted with sealable sampling ports. Cores were transported carefully back to the laboratory where they were incubated in an environmental chamber at *in situ* temperature in the dark (to replicate ambient light levels). At each site unfiltered bottom water was collected in 50-L polyethylene carboys covered with black plastic bags to exclude light. Bottom water was collected using a clean pumping system consisting of a Kynar® sampling tube connected to an all-plastic air-operated sampling pump (Wilden). The inlet end of the sampling

tubing was attached to a PVC-encased weight, which was lowered to the desired sampling depth using a nylon rope attached to the tubing.

Before beginning a flux experiment (typically within 24 h of return to the laboratory), the water overlying the cores was flushed using a peristaltic pump with ~3 volumes of unfiltered bottom water which was incubated at the same light and temperature conditions as the cores. The water level in the cores was adjusted to 8-13 cm above the sediment surface, with a slight space left between the overlying water level and the core top. The volume of water overlying the core was approximately 1.2 to 1.9 L. Filtered air was gently bubbled into the water overlying the core using small diameter Teflon tubing inserted through the cover plate. The air gently mixed the water and maintained its O_2 concentration at near-ambient levels, with negligible loss of CO_2 (Burdige and Homstead, 1994).

Samples of water overlying the core were removed as a function of time (generally 4-6 times over the course of 1 week) using a peristaltic pump fitted with trace-metal clean C-flex tubing and plastic connectors. Samples were filtered during collection through Meissner 0.2 μm polyethersulfone filter cartridges. Withdrawn volumes from the cores were replaced with equal volumes of bottom water. Analyte concentrations in the bottom estuary water used to recharge the cores were also monitored during the course of the experiment. Total dissolved Zn samples were stored in high-density polyethylene (HDPE) bottles and acidified to pH ~ 2 with ultrapure HCl (Fisher Optima) to prevent absorption of dissolved metal onto the bottle walls. Speciation samples were stored frozen (-20 °C) in fluorinated HDPE (FHDPE) bottles.

All sampling tubing, filters, and bottles were rigorously acid-washed using conventional trace metal techniques (Bruland, 1980) and all sample manipulations were carried out within laminar flow clean hoods equipped with HEPA-filtered air.

Total Dissolved Zn

Zinc was preconcentrated using an organic solvent extraction with microtechniques described by Rivera-Duarte and Flegal (1996). The method uses ammonium pyrrolidinedithiocarbamate (APDC) and diethylammonium diethyldithiocarbamate (DDDC) to complex the Zn ion. The complex was extracted into an organic solvent and then the organic complexes decomposed with acid to retrieve the metal ions. This procedure was performed in triplicate for each sample.

UV irradiation (1.2 kW Hg-arc lamp) of each sample was performed for 6 hours prior to use to destroy interfering organics. Due to volume limitation, aliquots (10 mL) of irradiated, acidified sample were diluted to 50 mL with Milli-Q water (Millipore; $\geq$ 18 MΩ cm^{-1}), and transferred to a Teflon separatory funnel. The pH of the solution was raised to between 4.0 and 4.5 with 5 M ultrapure NH_4OH (Fisher Optima). Solutions were then buffered with ammonium acetate buffer (400 μl) to a pH of approximately 4.5. A solution (250 μl) containing 1.5% each of APDC and DDDC in 1% NH_4OH was added. The APDC/DDDC solution was prepared daily. Prior to use ammonium acetate buffer and APDC/DDDC solutions were cleaned successively (six times) with ultrapure chloroform (Fisher Optima) extractions and ultrapure chloroform was cleaned with successive (10 times) extractions with dilute (pH 2) HCl. Sample solutions were extracted twice with 2 mL of ultrapure chloroform. The mixture was shaken vigorously for two minutes and the phases were allowed to separate within the funnel for five

minutes. The chloroform phases were combined into Teflon vials and evaporated on a hot plate (~30^{o}C). A volume of 1 mL of ultrapure 1 M HNO_3 (Fisher Optima) was then added to the vial, which was immediately capped. After another hour of heating, the vial was allowed to cool and the solution was diluted 10 fold. All extraction and heating processes were conducted in an exhausted HEPA laminar flow class 100 clean hood. Total dissolved Zn concentrations in the extracted samples were quantified by standard additions on a Perkin Elmer 5100 PC Atomic Absorption Spectrometer equipped with a 5100ZL Zeeman Furnace Module and AS 70 autosampler.

Blanks for the analysis of Zn were determined by extracting previously extracted samples. The average blank concentration was 8.4 ± 4.6 nM (n=21). The detection limit was 9 nM based on two times the standard deviation of the blanks. In order to verify the accuracy and precision of the TDZn method, the standard reference material, SLRS-4, was analyzed. Using this method, TDZn in SLRS-4 was 15 ± 2 nM (n=14), which was within the acceptable range of the certified value (14.2 ± 1.5 nM).

Zn Speciation Determinations

The speciation of dissolved Zn was determined using competitive ligand equilibrium-cathodic stripping voltammetry (CSV-CLE). This method allows quantification of Zn-complexing ligands, conditional stability constants of the Zn-ligand complexes, and speciation of Zn; i.e., the concentrations of organic complexes, inorganic complexes, and free hydrated ion (Ruzic, 1982). The CSV-CLE method involves establishment of a competitive equilibrium between Zn, Zn-complexing ligands naturally present in the sample, and a competing organic ligand, ammonium 1-pyrrolidinedithiocarbamate (APDC), added to the sample (van den Berg, 1985; Donat

and Bruland, 1990). The competitive Zn ligand, APDC, is capable of detecting ligands with K^{cond} of 10^7-10^{10}. This ligand was chosen because it is the only one currently available for electroanalytical speciation studies of Zn.

Samples were buffered at ambient pH using 1 M HEPPS, N-(2-hydroxyethyl)piperazine-N'-(3-propanesulfonic acid) (SigmaUltra), to a final buffer concentration of 0.01 M for each sample aliquot of 5.5 mL. Aliquots were added to two series of twelve 30 mL FEP-Teflon bottles. These FEP-Teflon bottles were soaked in warm (≈ 50 °C) concentrated HNO_3 for two weeks, rinsed with deionized water, and soaked for one week in dilute (pH 2) HCl (Fisher Trace Metal grade). The first bottle in each series was unspiked and the remaining pre-equilibrated bottles were spiked with incrementally increasing concentrations of Zn and allowed to establish a new equilibrium for 12 h. Concentrations of Zn additions ranged from 0 nM to 725 nM. Samples were transferred to FEP-Teflon cups prior to analysis and mounted onto the hanging mercury drop electrode (HMDE) cell stand, with a magnetic stirrer placed underneath. The sample was purged for 4 min with ultrapure N_2 (bubbled through Milli-Q water to minimize sample evaporation) to eliminate oxygen, which could cause a background signal that interferes with the analysis. The magnetic stirrer was set to a slow stirring rate (400 rpm) during purging after which sample stirring was stopped. Zn complexes were deposited at the HMDE that was set to deliver a medium drop size. The applied potential of -0.3 V was set for a deposition time of 20 seconds. This short deposition time was used to minimize surfactant effects as seen for Cu speciation measurements (Shank 2003). A negative-going potential scan was then applied and the reduction current from the Zn bound to added competing ligand under the newly established equilibrium

conditions was measured as a function of potential using differential pulse voltammetry. The voltammetric analyzer settings and reagent concentrations used for analysis are shown in Table 1. During analysis the added ligand outcompetes natural ligands and the titration curve becomes linear. Zinc speciation determinations were performed using an Autolab PGSTAT12 potentiostat coupled to an E.G. & G. Princeton Applied Research (PAR) model 303A HMDE and model 305 magnetic stirrer.

Speciation Calculations

The following calculations describe the relationships used to determine Zn speciation parameters (Ruzic,1982; van den Berg, 1985; Donat and Bruland, 1990). The mass balance of total dissolved Zn (TDZn) in the sample is:

$$[TDZn] = [Zn'] + [Zn - L]$$

where [*Zn-L*] is the concentration of Zn complexed by organic ligands and [*Zn'*] is the concentration of inorganic Zn. Major inorganic species include [Zn^{2+}], [$ZnCl^{+}$], [$Zn(CO_3)_2^{2-}$], [$ZnOH^{+}$], and [$ZnSO_4$] (Stanley and Byrne, 1990). The conditional stability constant of Zn-L with respect to free Zn^{2+} is:

$$K^{cond} = \left(\frac{[Zn - L]}{[Zn^{2+}] \quad [L']} \right)$$

where *L'* is the concentration of ligands not bound to the metal. The total Zn-complexing ligand concentration, L_T, is given by:

$$L_T = [L'] + [Zn - L]$$

Voltammetric Analyzer Settings	
Potentiostat	Autolab PGSTAT12
HMDE Stand	PAR Model 303A HMDE
Voltammetric Method	Differential Pulse
Deposition Potential	-0.3 V
Deposition Time	20 sec.
Re-oxidation Potential	-0.8 V
Re-oxidation Time	20 sec.
Equilibration Time	0 sec.
Stirring	Slow
Initial Potential	-0.95 V
End Potential	-1.175 V
Step Potential	0.0021 V
Amplitude	0.05 mV
Purge with N_2	4 min.

Table 1. Voltammetric analyzer settings for the analysis of Zn speciation.

The following relationship can then be used to calculate values for L_T and K^{cond} (van den Berg, 1985).

$$\left(\frac{[Zn^{2+}]}{[Zn-L]}\right)=\left(\frac{[Zn^{2+}]}{L_T+\frac{1}{(K^{cond}*L_T)}}\right)$$

For a one ligand system, a plot of $\left(\frac{[Zn^{2+}]}{[Zn-L]}\right)$ as a function of $[Zn^{2+}]$ is linear, where the slope is $\left(\frac{1}{L_T}\right)$ and the y-intercept is $\left(\frac{1}{(K^{cond}*L_T)}\right)$.

The measured current, i_p, is caused by the reduction of Zn^{2+} at the electrode surface after adsorption of the Zn-PDC complexes. Free Zn^{2+} concentration is related to i_p through:

$$[Zn^{2+}]=\left(\frac{i_P}{(S*\alpha')}\right)$$

where S is the slope of the linear portion of the Zn titration curve occurring after the zinc-complexing ligands have been saturated with Zn and α' is the overall side reaction coefficient for Zn:

$$\alpha'=\alpha_{Zn}+\alpha_{ZnPDC}$$

In this expression, α_{Zn} is the inorganic side reaction coefficient for zinc and α_{ZnPDC} is the side reaction coefficient for Zn complexed with PDC:

$$\alpha_{ZnPDC}=K^{cond}[APDC']$$

where [*APDC'*] is the concentration of APDC not complexed by Zn^{2+}. Since the concentration of APDC not complexed by Zn^{2+} is approximately equal to total APDC; it is assumed they are equal. These side reaction coefficients are calculated from literature values. van den Berg (1985) calculated log K^{cond} and α_{Zn} at various salinities. In the present work the α_{ZnPDC} was determined to be 1.0, α_{Zn} varied from 1.5 to 2.0 and α' varied from 2.58 to 2.99. The strength of the ligands can be detected using this method have conditional stability constants, K^{cond}, ranging from approximately $10^{7.0}$ to 10^{10}.

Flux Calculations

TDZn and Zn-complexing ligand fluxes were determined by linear least squares fitting of corrected concentrations vs. time. Measured concentrations of these parameters were corrected for changes occurring in the recharge water during incubation and for the addition of recharge water during sampling. Corrected concentrations were then converted into benthic fluxes by accounting for the volume of overlying water and sediment surface area within each core. Fluxes were considered to be significant if the results from the linear regression of corrected concentrations vs. time produced a statistically significant fit ($p < 0.05$). When the flux calculations yielded $p > 0.05$ then the fluxes were reported as having a zero net flux. Negative values indicate a flux into sediments from overlying waters and a positive value indicates a flux out of the sediment.

Humic Substances Extraction

Humic substances were extracted from a freshwater sample (salinity = 0) using Sep Pak® C_{18} cartridges as described in Kieber et al. (1999). The sample was collected at Navassa (NAV) on June 18, 2003 (Figure 1) and filtered through Meissner 0.2 μm polyethersulfone filters using a peristaltic pump. The filtered sample was loaded onto C_{18}

cartridges that were previously washed with 20 mL of HPLC grade acetonitrile followed by 20 mL of Milli-Q. The sample was pulled through C_{18} cartridges (250 mL sample per C_{18} cartridge) at a flow rate of approximately 3 mL min^{-1}. Humics were eluted off with 2 mL of acetonitrile into a 100 mL conical flask and concentrated to dryness under reduced pressure (Buchi Rotavapor, Model R-3000, Switzerland). Any traces of water were then removed under vacuum. A humic solution was prepared to mimic typical salinity and humic concentrations in the Cape Fear Estuary. Gulf Stream water collected ≈100 km from the coast of North Carolina (salinity ≈ 35) was filtered through 0.2 μm Millipore Isopore membrane filters and UV-irradiated. The seawater was diluted with an appropriate volume of Milli-Q to create a solution with a salinity of ≈ 10 and 20 mg of humic extract dissolved in 1 L solution. This humic solution was then analyzed for TDZn and Zn-complexing ligand. An analogous blank solution without humics was also analyzed.

RESULTS AND DISCUSSION

Total Dissolved Zn and Zn-Complexing Ligands

Concentrations of TDZn and Zn-complexing ligands (Zn-L) in bottom waters of the Cape Fear estuary (CFE) were determined by analyzing the recharge water collected during core incubation experiments at time point T0 (Table 2). Overall TDZn concentrations ranged from ND to 44 nM in the upper estuary and ND to 61 nM in the lower estuary. Zn-L concentrations ranged from 10 to 102 nM in the upper estuary and non-detectable (ND) to 67 nM in the lower estuary. Concentrations of TDZn are much lower than the EPA water quality criterion for seawater (1. 2 μM Zn; EPA, 2002). Earlier work in the CFE found similar TDZn and Zn-L concentrations at similar estuarine

[TDZn] (nM)			
Season	**Sampling Date**	**Station 1**	**Station 2**
Fall	**November 29, 2000**	19 ± 10	23 ± 1
	October 29, 2001	24 ± 3	61 ± 13
Summer	**June 26, 2001**	30 ± 12	43 ± 6
	June 28, 2002	ND	ND
Spring	**April 23, 2001**	44 ± 29	ND
Winter-Spring	**March 7, 2002**	31 ± 2	ND

a.

[Zn-L] (nM)			
Season	**Sampling Date**	**Station 1**	**Station 2**
Fall	**November 29, 2000**	102 ± 8	25 ± 6
	October 29, 2001	10 ± 4	67 ± 18
Summer	**June 26, 2001**	38 ± 23	44 ± 7
	June 28, 2002	12 ± 3	ND
Spring	**April 23, 2001**	31 ± 18	ND
Winter-Spring	**March 7, 2002**	101 ± 11	ND

b.

Table 2. Concentrations of (a) total dissolved Zn and (b) Zn-complexing ligands from bottom water at time point T0 from Station 1 and Station 2 at each sampling time (ND = non-detectable).

sites (Table 3). O'Connell (1999) observed TDZn concentrations throughout the estuary ranging from 15 to 90 nM. MacGillivray (2002) found TDZn concentrations from 5 to 10 nM and Zn-L concentrations from 36 to 96 nM in the lower estuary.

Values reported for the CFE are similar to previous work found in other East Coast estuaries, such as Chesapeake Bay and Narragansett Bay (Table 3). Henry (1996) found concentration ranges of TDZn and Zn-L of 3 to 39 nM and 12 to 54 nM, respectively, in Chesapeake Bay. Kozelka and Bruland (1998) found TDZn concentrations of 16 to 72 nM and Zn-L concentrations of 11 to 48 nM in Narragansett Bay. In contrast, Manila Bay, a relatively more impacted estuary, had TDZn levels ranging from 2 to 147 nM and Zn-L ranging from 2 to 123 nM, where elevated levels occurred near point sources (Vesasquez et. al., 2002). The higher concentrations observed in impacted estuaries suggests the possibility of anthropogenic inputs.

Variability of TDZn and Zn-L was observed between seasons and sampling locations in the CFE. Seasonal variability was observed in the lower estuary (Station 2) where higher concentrations occurred in the fall compared to the spring and winter-spring. No seasonal variability occurred in the middle estuary (Station 1). Concentrations of TDZn and Zn-L were also much greater in the spring and winter in the middle estuary compared to the lower estuary.

There was no significant correlation observed between TDZn and Zn-L when all data were plotted together (Figure 2). However, when the data were divided between stations, a significant correlation occurred at Station 2 (Figure 3b; $R = 0.996$, $p \leq 0.01$) while there was no correlation at Station 1 (Figure 3a). Station 2 concentrations of TDZn and Zn-L were tightly coupled. This behavior was also observed in the Scheldt Estuary,

Total Dissolved Zinc and Zinc-Ligand Concentrations			
Location	**[TDZn] (nM)**	**[Zn-L] (nM)**	**Reference**
Chesapeake Bay (U.S.A.)	3-39	12-54	Henry, 1996
Galveston Bay (U.S.A.)	2-15	-	Warnken et al., 2001
Humber Estuary (U.K)	91-275	46-275	Gardner, 1999
Manila Bay (Philippines)	2-147	2-123	Vesasquez et al., 2002
Narragansett Bay (U.S.A.)	16-72	11-48	Kozelka and Bruland, 1998
Sabine (U.S.A.)	10-15	15-20	Benoit et al., 1994
San Francisco (U.S.A.)	3-29	-	Sanudo-Wilhelmy et al., 1996
Scheldt Estuary (Netherlands)	15-230 3-260	22-220 -	van den Berg et al., 1987 Zwolsman, 1997
Tamar Estuary (U.K.)		40-160	van den Berg et al., 1986
Cape Fear Estuary	15-90 5-10 2-61	- 36-96 ND-102	O'Connell, 1999 MacGillivray, 2002 This study

Table 3. Concentrations of TDZn and Zn-complexing ligands in selected estuarine environments.

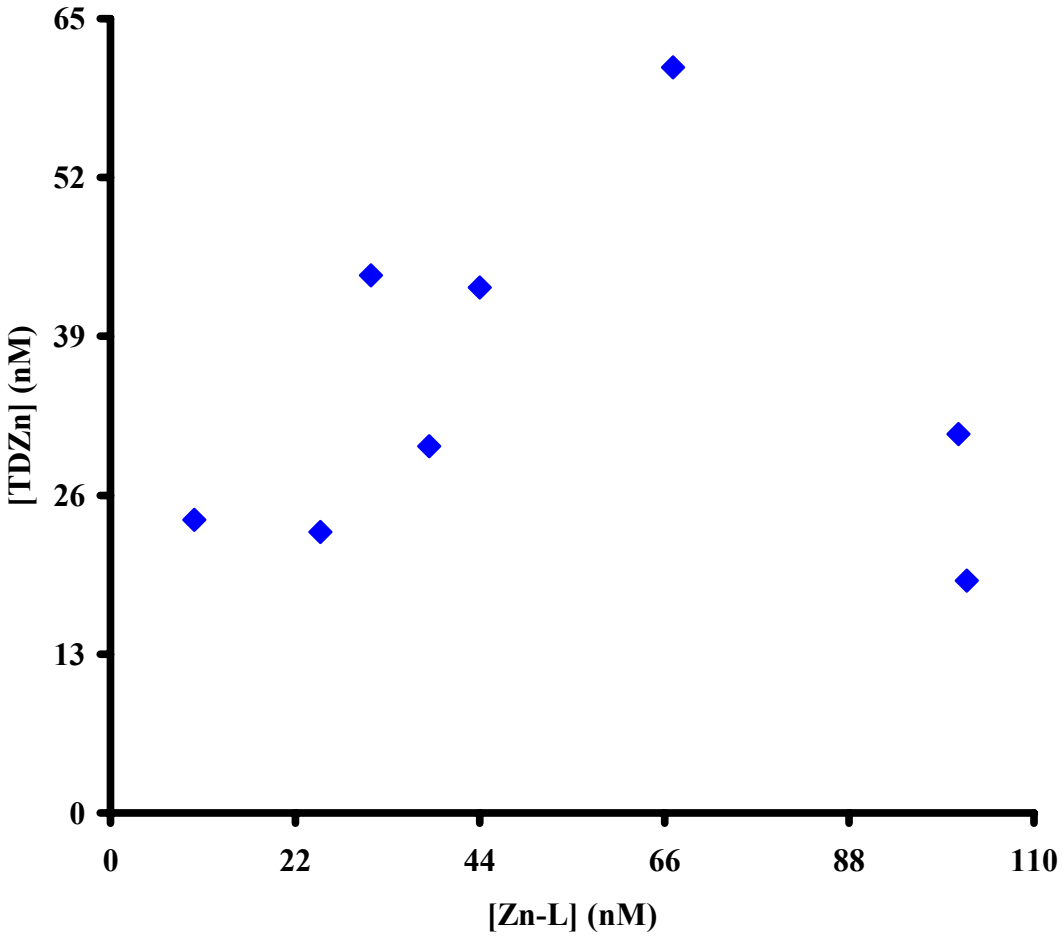
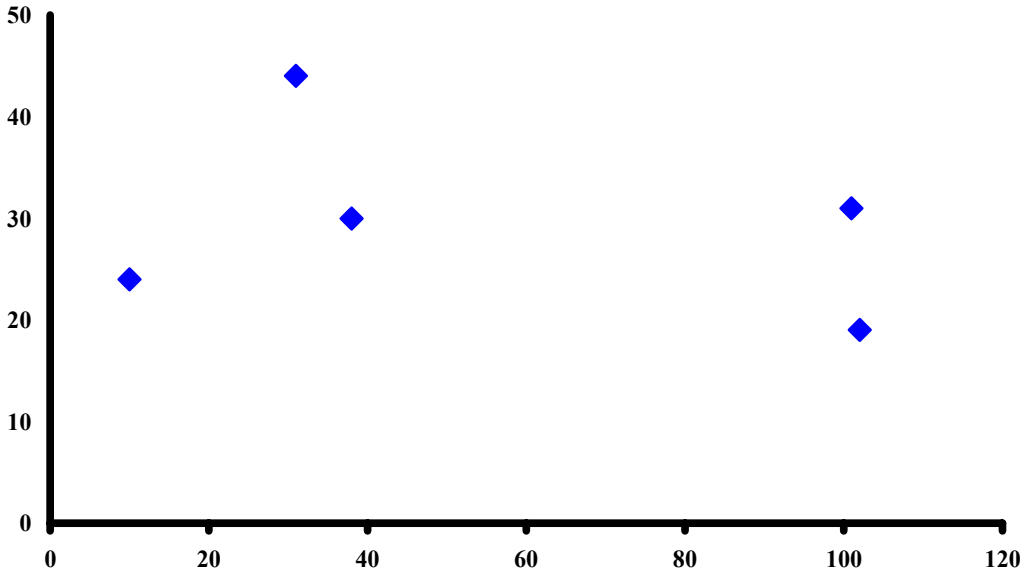
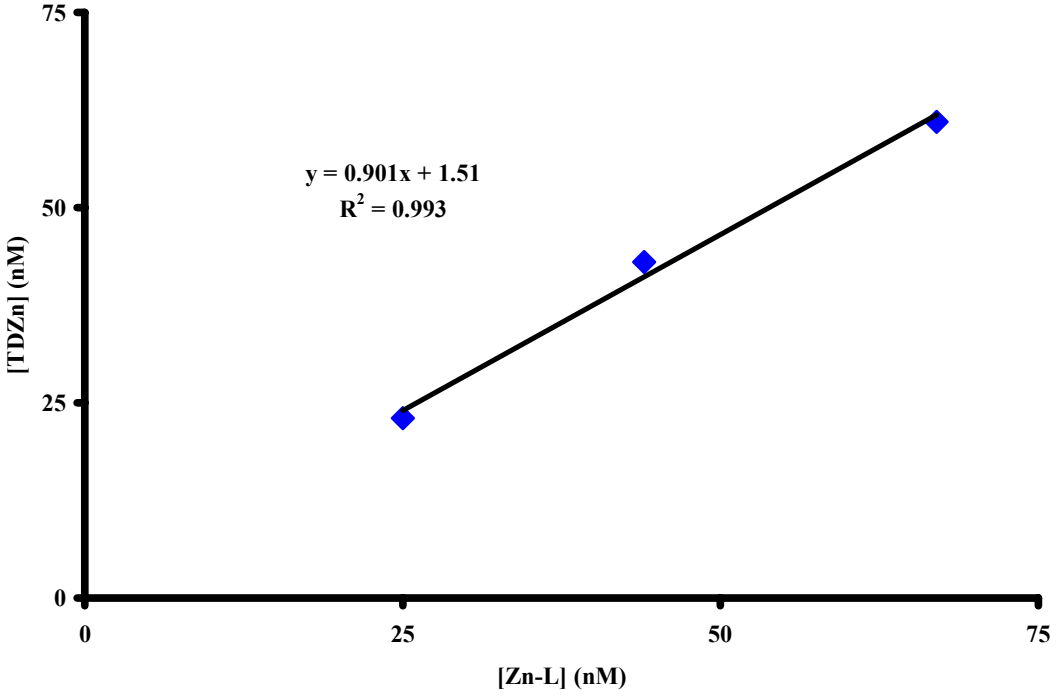


Figure 2. Relationship between TDZn and Zn-complexing ligands from bottom waters at both sampling stations at all sampling times. There is no statistically significant correlation between the two parameters ($p > 0.05$).



a.



b.

Figure 3. Relationship between TDZn and Zn-complexing ligands from bottom waters at (a) Station 1 (no significant correlation; $p > 0.05$) and (b) Station 2 (significant corrrelation; $p \leq 0.01$).

where the TDZn concentrations (15 to 230 nM) were relatively similar to those of Zn-L (22 to 220 nM). In contrast, Station 1 concentrations were more variable, with Zn-L concentrations greater than TDZn concentrations in 4 of the 6 experiments. In 2 of the 6 experiments, Zn-L concentrations were less than TDZn, suggesting that TDZn can titrate out relatively strong ligands leaving the remaining Zn as either weakly complexed Zn or inorganic Zn. This behavior was observed in a more impacted British estuary, the Humber, in which the TDZn concentrations (91 to 275 nM) were consistently greater than Zn-L (46 to 275 nM). Calculations of organically complexed Zn, using the approach described in Ellwood and van den Berg (2000) showed 25 to 84% of the TDZn occurring in CFE as organic complexes. The degree of complexation of Zn in the CFE is generally much lower than that of Cu, of which >99.9% exists as complexed species throughout the estuary (Shank, 2003). This differing behavior is mainly a consequence of the much weaker nature (lower K^{cond}) of the Zn-L complexes, as well as the comparatively lower concentrations of Zn ligands relative to TDZn in the CFE. A significant fraction of dissolved Zn in the estuary therefore exists in inorganic or weakly organically complexed forms, whereas dissolved Cu exists predominantly as very strong organic complexes.

Organic rich estuaries such as the Cape Fear may contain relatively high concentrations of metal-complexing ligands, given DOC concentrations typically range from 200-800 μM (Avery et al., 2003). Avery et al. (2003) reported the conservative nature of DOC in the Cape Fear estuary; these observations are supported by data collected in this study (Table 4; Figure 4; $R = 0.967$, $p \leq 0.001$). Shank (2003) determined that concentrations of Cu-complexing ligands were highly correlated with DOC concentrations and suggested that concentrations of Cu-complexing ligands could

Sampling Date	Salinity	DOC (μM C)	River Flow (m^3/s)
Station 1			
November 19, 2000	18	627	77
April 23, 2001	17	552	52
June 26, 2001	7	886	110
October 29, 2001	25	366	29
March 7, 2002	10	670	81
June 28, 2002	25	379	24
Station 2			
November 19, 2000	23	440	77
April 23, 2001	27	289	52
June 26, 2001	23	492	110
October 29, 2001	29	277	29
March 7, 2002	22	397	81
June 28, 2002	32	229	24

Table 4. Summary of salinity, DOC, and river flow of Station 1 and Station 2 during each sampling time.

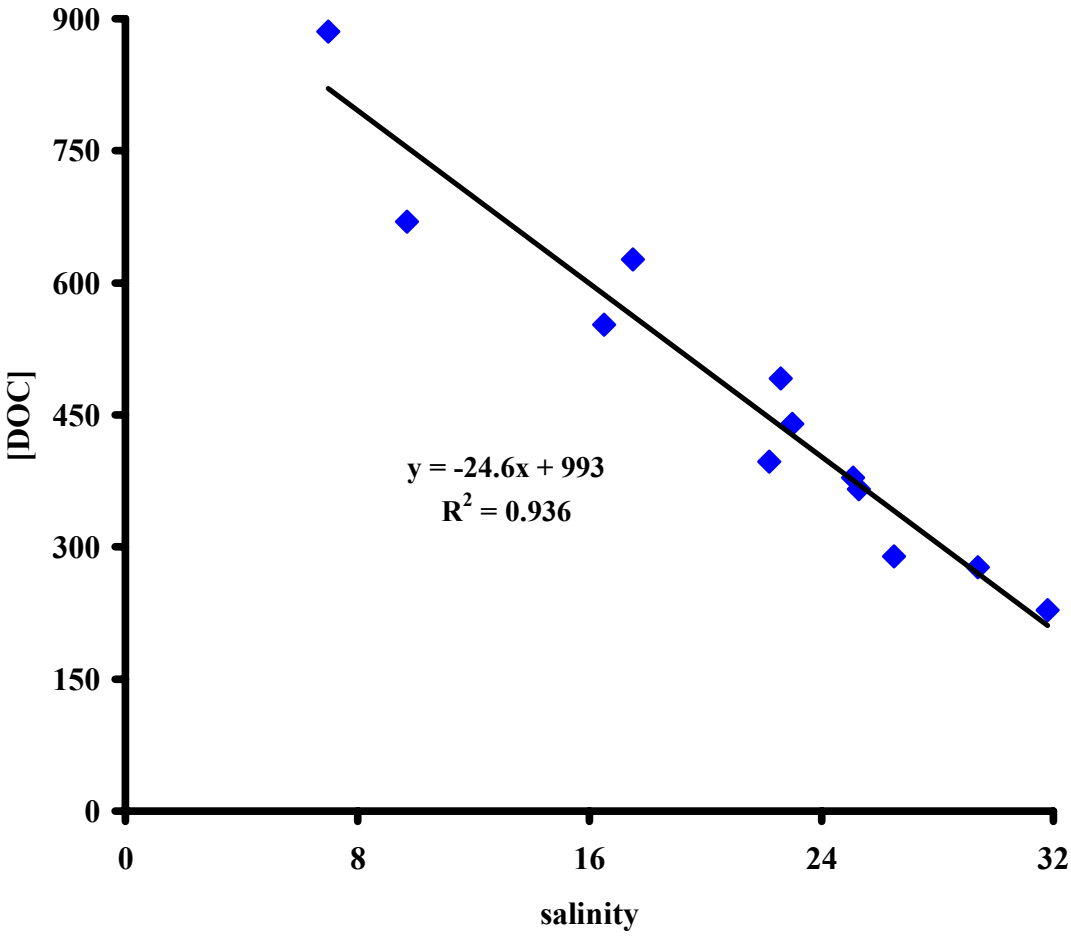
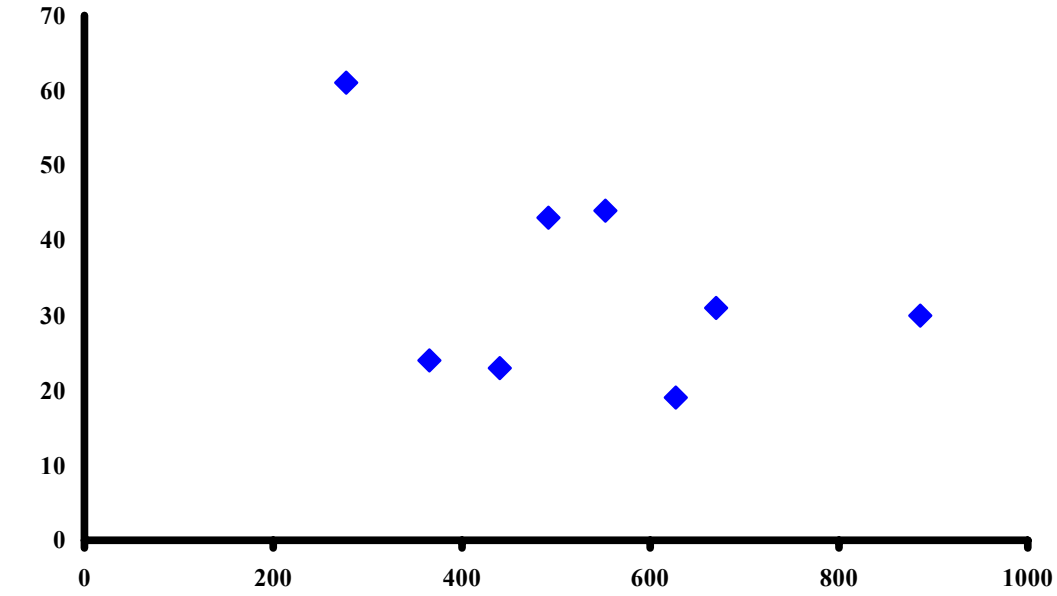


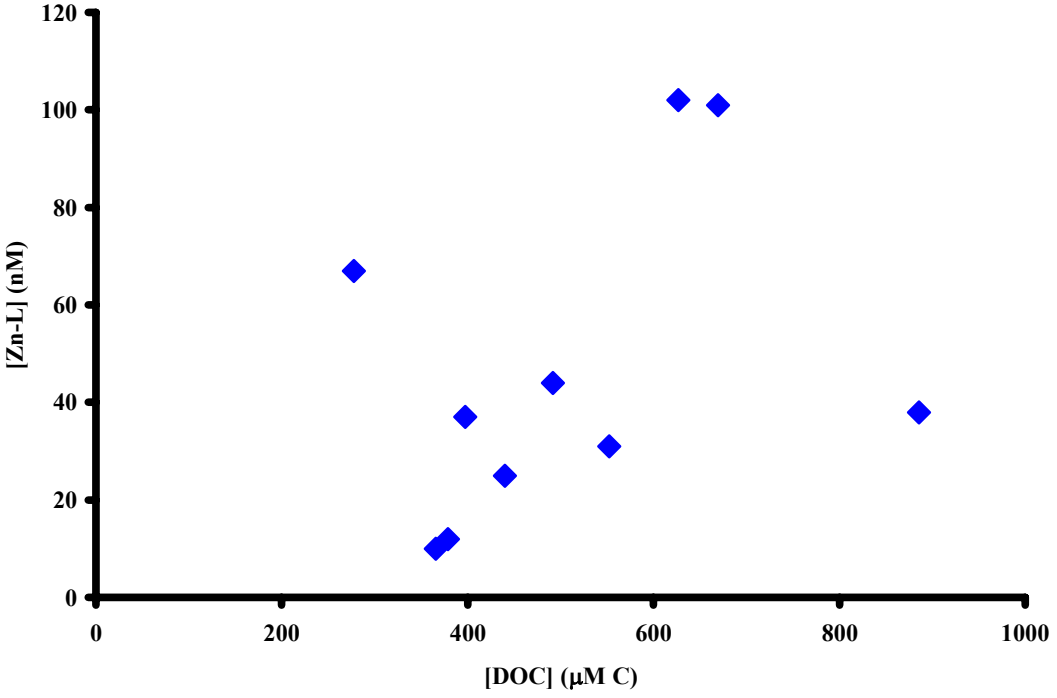
Figure 4. Relationship between DOC concentrations and salinity from bottom waters at both sampling stations at all sampling times (significant correlation; $p \leq 0.001$).

be estimated from the DOC concentrations. The significant correlation between DOC and strong Cu-complexing ligands indicates that a small but significant fraction of DOC consists of Cu-complexing ligands. Zn-complexing ligand concentrations were plotted as a function of DOC in order to determine if a significant correlation could also be observed. No statistically significant correlations occurred between DOC and TDZn or Zn-complexing ligands (Figure 5). This suggests that Zn-complexing ligands comprise a more variable proportion of the DOC pool in the CFE, relative to the fraction of DOC that consists of strong Cu-complexing ligands. It is also possible that there is a class of Zn-complexing ligands that co-varies with DOC, but is too weak to be detected by the current method.

Concentrations of DOC in the CFE were positively correlated to river flow (Table 4; Figure 6; $R = 0.735$, $p \leq 0.01$). This relationship has been observed for other estuaries along the southeast U.S. coast (Moran et al., 1999) and suggests that DOC is flushed from upland area and adjoining soils during times of high rainfall and subsequent runoff. The impact of river flow was also examined for TDZn and Zn-complexing ligands using USGS (United States Geological Survey) river flow data from the Cape Fear River (available at http://waterdata.usgs.gov/nc/nwis). River flow was calculated by taking an average of the daily mean stream flow (ft^3/s) for six days before the sampling date along with the sampling date and converted to (m^3/s). The average was calculated for one week to correspond with the approximate residence time of water in the estuary. There was no statistically significant correlation between TDZn and Zn-complexing ligands and river flow (Figure 7). This lack of correlation suggests the importance of local inputs of zinc and ligands that are not significantly affected by river stage. This behavior differs from



a.



b.

Figure 5. Relationship between (a) TDZn and (b) Zn-complexing ligands and DOC from bottom waters at both sampling stations at all sampling times. Both plots show no statistical significance ($p > 0.05$).

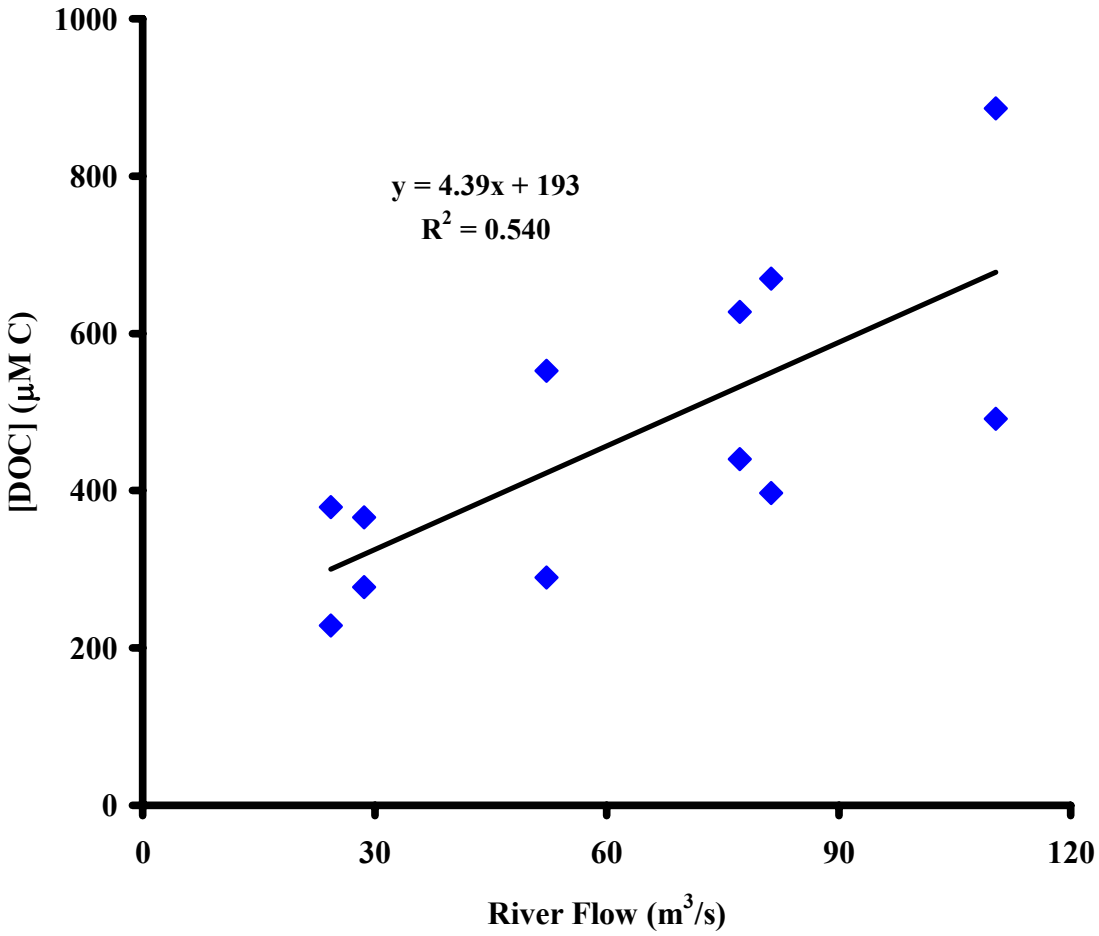
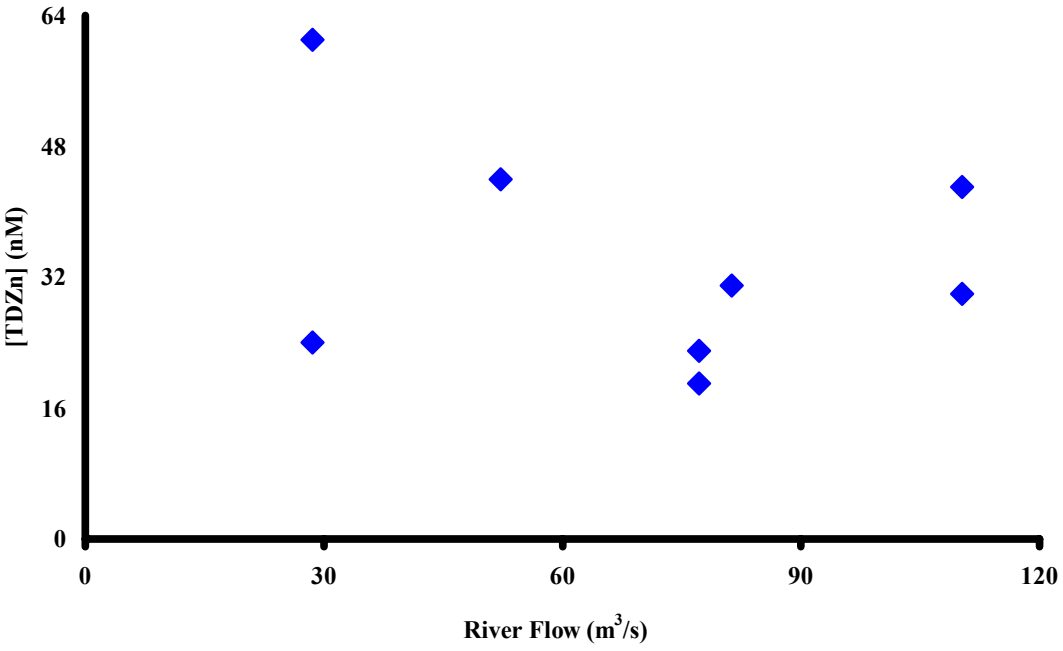
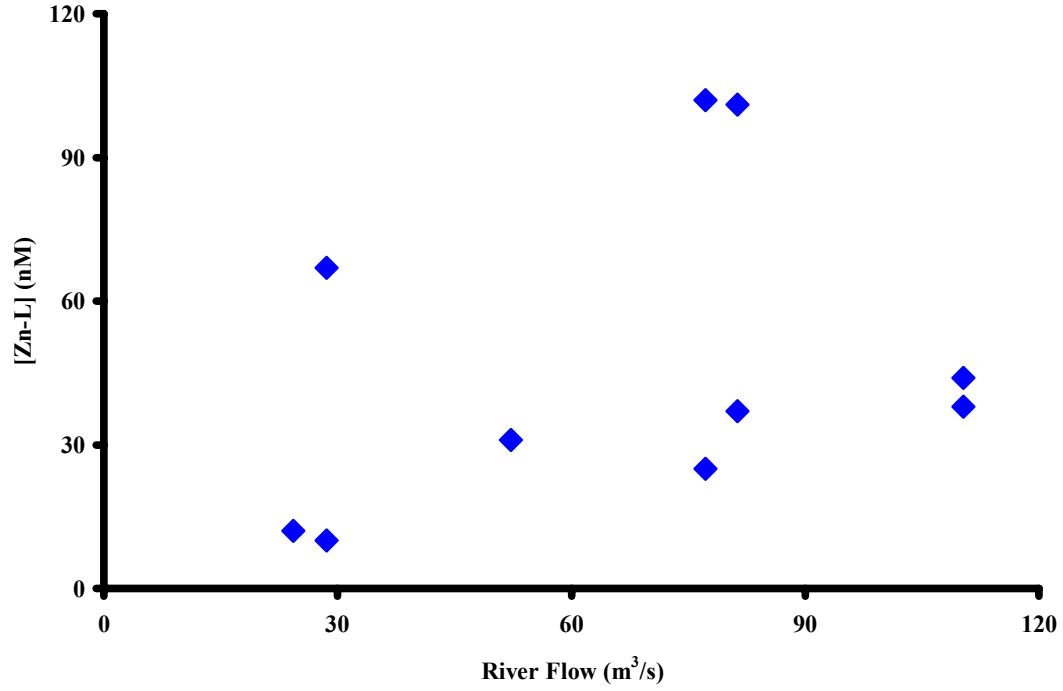


Figure 6. Relationship between DOC concentrations and river flow, showing a statistically significant correlation ($p \leq 0.01$).



a.



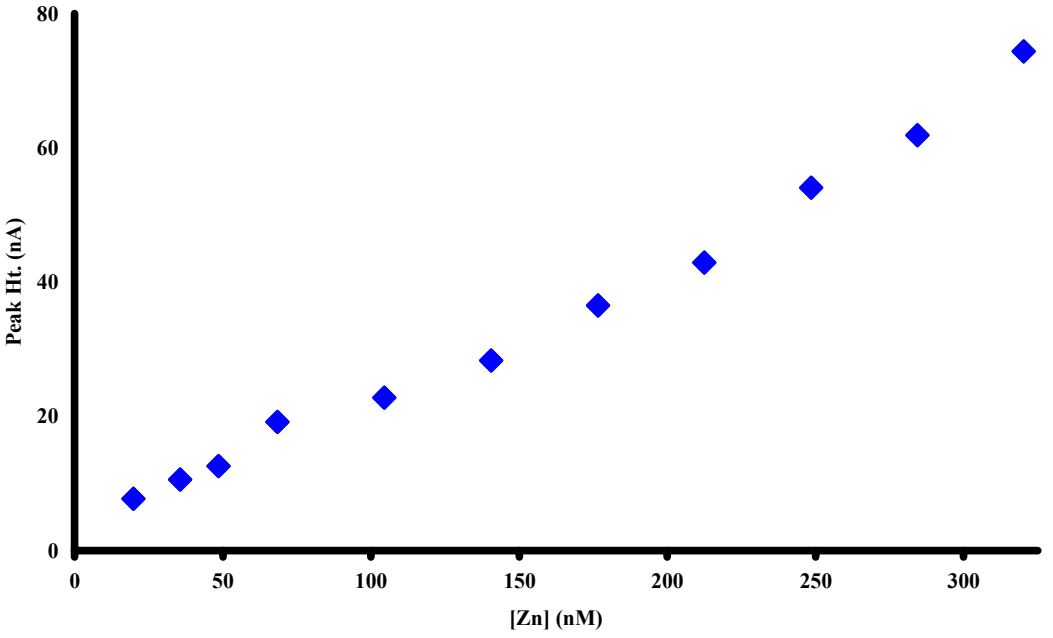
b.

Figure 7. Relationship between (a) TDZn and (b) Zn-complexing ligands and river flow from bottom waters at both sampling stations at all sampling times. Both plots show no statistical significance ($p > 0.05$).

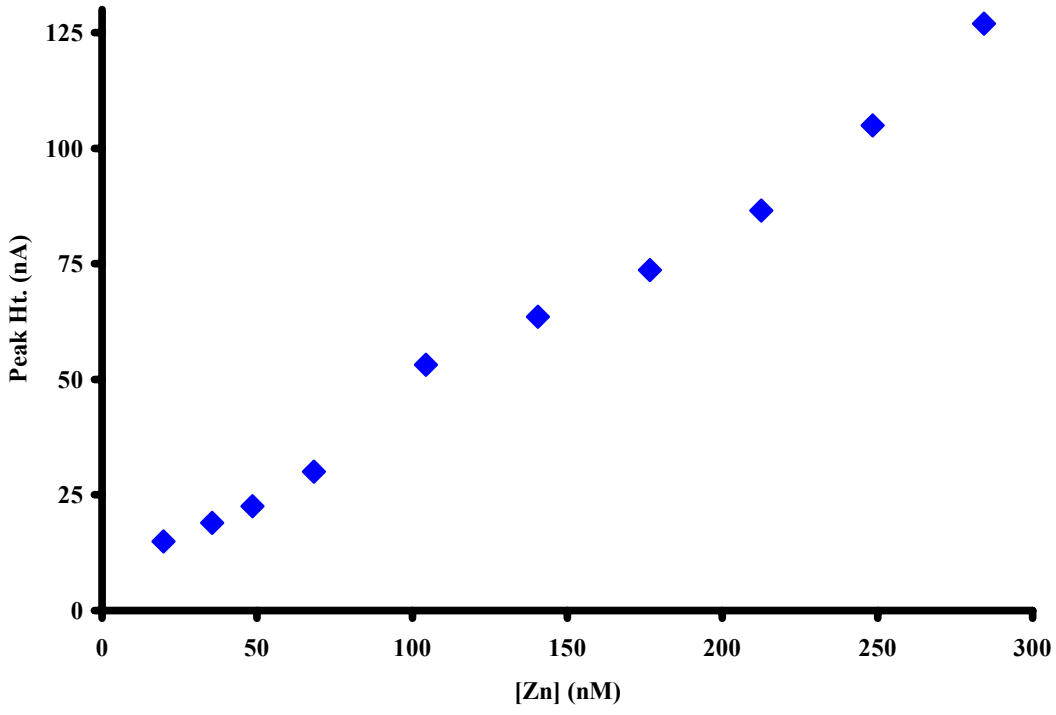
what has been observed for Cu-complexing ligands in the CFE because the fraction of DOC that strongly complexes Cu increased (with total DOC) with increased river flow (Shank, 2003).

Role of Humics as Zn-Complexing Ligands

Since it is known that a large fraction of DOC in the CFE consists of humic substances (Avery et al., 2003) and that humics extracted from the CFE are very effective strong Cu chelators (Shank, 2003), it was important to examine the role of humics as Zn chelators. Humics isolated from Cape Fear freshwater using solid phase C_{18} extraction were used to prepare 20 mg/L solutions (typical of the middle CFE) in UV-irradiated seawater diluted to a salinity of 10. As shown in Figure 8a, titrations of the humic solutions did not produce an initially supressed response on the titration curve, indicating that humics do not complex Zn or only weakly complexes Zn at a level less than that detectable by the competitive ligand, APDC. It is possible that the ambient TDZn titrated out a small concentration of relative strong Zn ligands, leaving remaining Zn as either more weakly complexed Zn or inorganic Zn. Blank solutions containing diluted UV-irradiated seawater of salinity 10 containing no added humics yielded titration curves very similar to solutions containing humics (Figure 8b). It is clear from these results that C_{18}-extractable humics are not a significant source of relatively strong Zn-complexing ligands in the CFE. In contrast to Zn, humic substances dominate the pool of strong Cu ligands in the CFE (Shank, 2003). Strong complexation of Cu with humics is indicated by the strong initial supressed response (Figure 9; data from Shank, 2003). These results suggest that the ligands complexed to Zn and Cu are different and factors favoring strong Cu complexation in the CFE do not necessarily favor Zn complexation.



a.



b.

Figure 8. Titration curves in the (a) presence and (b) absence of C_{18} extracted humic substances (20 mg/L) in UV-irradiated diluted seawater (salinity = 10).

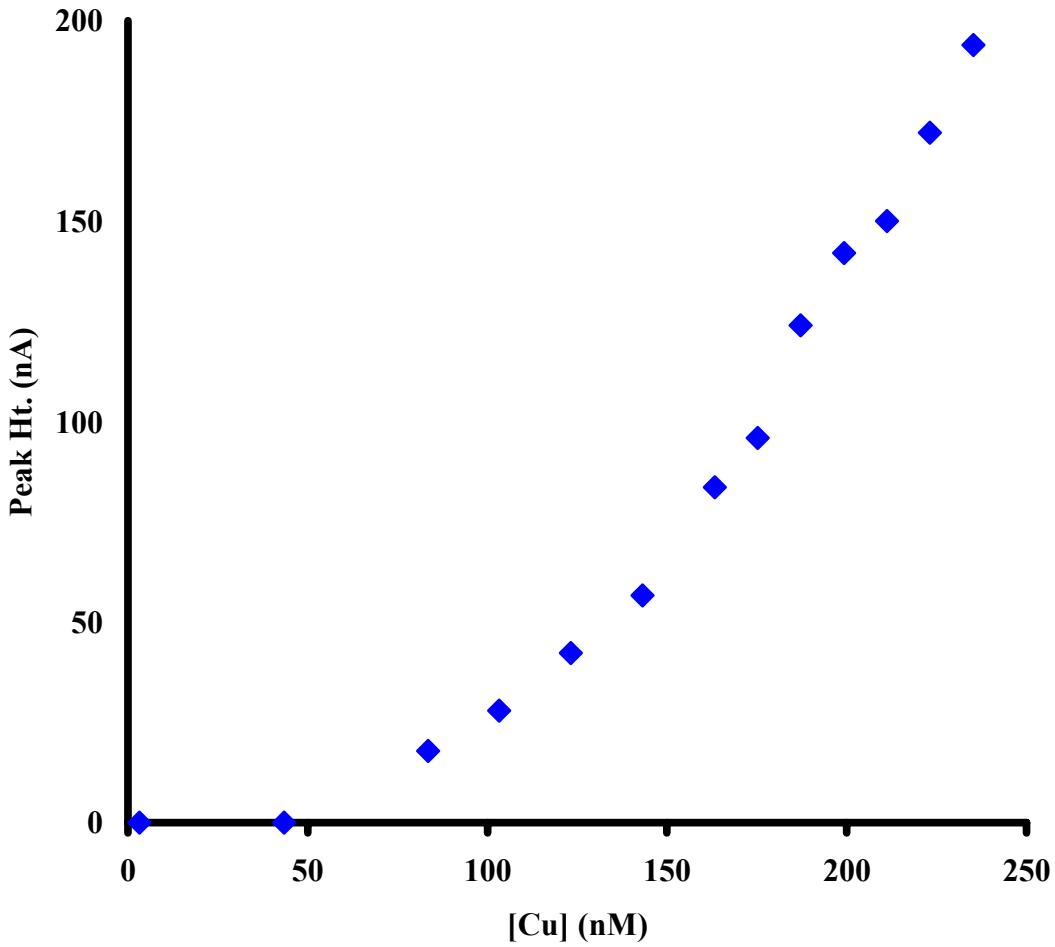


Figure 9. Titration curve showing complexation of Cu in a 19.5 mg/L solution of humic substances (extracted from the CFE) in UV-irradiated diluted seawater (salinity = 12). Data from Shank (2003).

Benthic Fluxes

In order to determine if sediments are a source of TDZn and Zn-complexing ligands to the CFE, a series of benthic flux experiments was conducted using a core incubation technique. Fluxes measured using the core incubation technique result from diffusive or bioturbation processes. Advective and turbulent processes and hydrostatic influences such as tidal pumping are not taken into account. Core incubation has the advantage of directly measuring net fluxes, including the effects of processes occurring at the sediment water interface.

Fluxes measured using this technique were considered to be significant if the results from the linear regression of corrected concentrations vs. time produced a statistically significant fit ($p < 0.05$). When flux calculations yielded $p > 0.05$, then fluxes were reported as a zero net flux. Negative values indicate a flux into sediments from overlying waters and a positive value indicates a flux out of the sediment.

Total Dissolved Zn Fluxes

Total dissolved Zn fluxes ranged from -1100 to 1800 nmol m^{-2} d^{-1} at both sampling stations over all sampling periods (Table 5). These fluxes bracket the -56 to 300 nmol m^{-2} d^{-1} found at Station 2 in the spring and summer in the CFE (MacGillivray, 2002). Byers (1999) observed TDZn fluxes measured using porewater profiles in the Elizabeth River (southern Virginia) ranging from -57 to 832 nmol m^{-2} d^{-1}. These comparable TDZn fluxes are interesting because both the Cape Fear and Elizabeth River estuaries are subject to inputs from industrial, municipal, and shipping activities. Fluxes of TDZn at different estuaries are summarized in Table 6. Large, impacted estuaries such as Galveston Bay and San Diego Bay show relatively large fluxes from the sediments

TDZn Fluxes (nmol m^{-2} d^{-1})			
Season	**Sampling Date**	**Station 1**	**Station 2**
Fall	**November 29, 2000** (n = 2)	0 1800 ($p \leq 0.02$)	1300 ($p \leq 0.02$) 0
	October 29, 2001 (n = 3)	0 0 0	0 0 -480 ($p \leq 0.05$)
Summer	**June 26, 2001** (n = 2)	-1100 ($p \leq 0.02$) 0	0 0
	June 28, 2002 (n = 3)	460 ($p \leq 0.02$) 520 ($p \leq 0.001$) 0	0 1000 ($p \leq 0.02$) 200 ($p \leq 0.02$)
Spring	**April 23, 2001** (n = 2)	0 200 ($p \leq 0.05$)	0 0
Winter-Spring	**March 7, 2002** (n = 3)	0 0 0	-160 ($p \leq 0.001$) 1200 ($p \leq 0.02$) 180 ($p \leq 0.001$)

Table 5. Total dissolved zinc fluxes for individual core measurements at Station 1 and Station 2 at each sampling date. Negative values indicate a flux into the sediment. Positive values indicate flux out of the sediment. Non-statistically significant fluxes are represented as "0" net flux. n = number of cores per sampling site. p = level of significance.

Total Dissolved Zn and Zn-Ligand Fluxes (nmol m^{-2} d^{-1})			
Location	**TDZn**	**Zn-L**	**Reference**
Elizabeth River	-57 to 898	144 to 1349	Byers, 1999
Galveston Bay	2400	-	Warnken et al., 2001
San Diego Bay	1500 to 31000	-	Anderson et al., 2001
Cape Fear Estuary	-56 to 300	-980 to 1200	MacGillivray, 2002
	-1071 to 1826	-1692 to 2850	This study

Table 6. Fluxes for TDZn and Zn-complexing ligands in different estuarine systems. Negative values indicate a flux into the sediment. Positive values indicate flux out of the sediment.

ranging from 2400 nmol m^{-2} d^{-1} and 1500 to 31000 nmol m^{-2} d^{-1} respectively. The higher TDZn fluxes from impacted estuaries may explain why higher TDZn concentrations were observed in the overlying waters of these estuaries (Table 2). The smaller fluxes from the CFE probably reflect a lower degree of contamination of the bottom sediments.

Statistically significant TDZn fluxes were observed in 12 of the 30 cores for all experiments, 9 of which were positive indicating a flux of TDZn to overlying waters from sediments. The TDZn flux of greatest magnitude (1800 nmol m^{-2} d^{-1}) measured during the entire project was a positive flux occurring during November 2000 at the upper estuary site. Overall, the sporadic nature of TDZn benthic fluxes suggests that sediments are not a significant source of TDZn to waters of the CFE, similar to the conclusion of MacGillivray (2002).

Zn Ligand Fluxes

Zn ligand fluxes ranged from -1700 to 2900 nmol m^{-2} d^{-1} for both stations over the sampling period (Table 7). These values are comparable to earlier work in the lower Cape Fear estuary showing ligand fluxes from -980 to 1200 nmol m^{-2} d^{-1} (MacGillivray, 2002). Interestingly, the Zn ligand flux of greatest magnitude (2850 nmol m^{-2} d^{-1}) occurred at the same time as the largest TDZn flux. Statistically significant Zn ligand fluxes were observed in only 8 of the 30 cores, of which 6 were positive fluxes. This indicates sediments are generally not a significant source of Zn complexing ligands to the water column. Measurements of Zn ligand fluxes in MacGillivary (2002) and in the present work are the only direct measurements of Zn-complexing ligand fluxes that have been reported.

Zn-L Fluxes (nmol m^{-2} d^{-1})			
Season	**Sampling Date**	**Station 1**	**Station 2**
Fall	**November 29, 2000** (n = 2)	2900 ($p \leq 0.02$) 0	140 ($p \leq 0.01$) 570 ($p \leq 0.001$)
	October 29, 2001 (n = 3)	0 1900 ($p \leq 0.01$) 2700 ($p \leq 0.001$)	0 0 0
Summer	**June 26, 2001** (n = 2)	0 0	0 0
	June 28, 2002 (n = 3)	0 0 0	2000 ($p \leq 0.02$) 0 0
Spring	**April 23, 2001** (n = 2)	0 -440 ($p \leq 0.02$)	0 0
Winter-Spring	**March 7, 2002** (n = 3)	0 0 -1700 ($p \leq 0.01$)	0 0 0

Table 7. Zn-ligand fluxes for individual core measurements at Station 1 and Station 2 at each sampling date. Negative values indicate a flux into the sediment. Positive values indicate flux out of the sediment. Non-statistically significant fluxes are represented as "0" net flux. n = number of cores per sampling site. p = level of significance.

Studies by Byers (1999) and van den Berg and Dharmvanij (1984) show estuarine sediment porewaters have relatively high concentrations of Zn-complexing ligands compared to overlying waters, suggesting that porewaters can be a source of Zn-complexing ligands. Using porewater profiles, Byers (1999) estimated Zn ligand fluxes in the Elizabeth River estuary of 144 to 1349 nmol m^{-2} d^{-1}. Even though porewater gradients should be predictive of diffusive fluxes, this approach does not account for processes occurring at the sediment water interface that may mediate exchange across the interface. Other inconsistencies may be related to disturbance of the diffusive boundary layer during sampling or as a result of removing the sediments. Thus, direct measurements of Zn ligand fluxes using the core incubation method may provide a more accurate estimate of benthic ligand fluxes than an indirect estimate using porewater profiles.

Comparison of Zn and Cu Speciation and Fluxes

Benthic fluxes of total dissolved Cu and Cu-complexing ligands were determined by Shank (2003) using the same core incubation technique at the same two stations used during this study (Table 8). Concurrent studies of Zn and Cu occurred during April 2001, June 2001, October 2001, and June 2002. No correlation was observed between benthic fluxes for TDZn and TDCu or Zn- and Cu-complexing ligands. Statistically significant TDCu fluxes occurred in 33% of the incubated cores and significant TDZn fluxes occurred in 35% of the cores. Cu-ligand fluxes were observed in 50% of the incubated cores and Zn-ligand fluxes were observed in 20% of the incubated cores. The majority of the cores showed neither a flux in nor out of the sediment. However, when there was a significant flux of Zn-L (Station 1, October 2001) it did not coincide with a significant

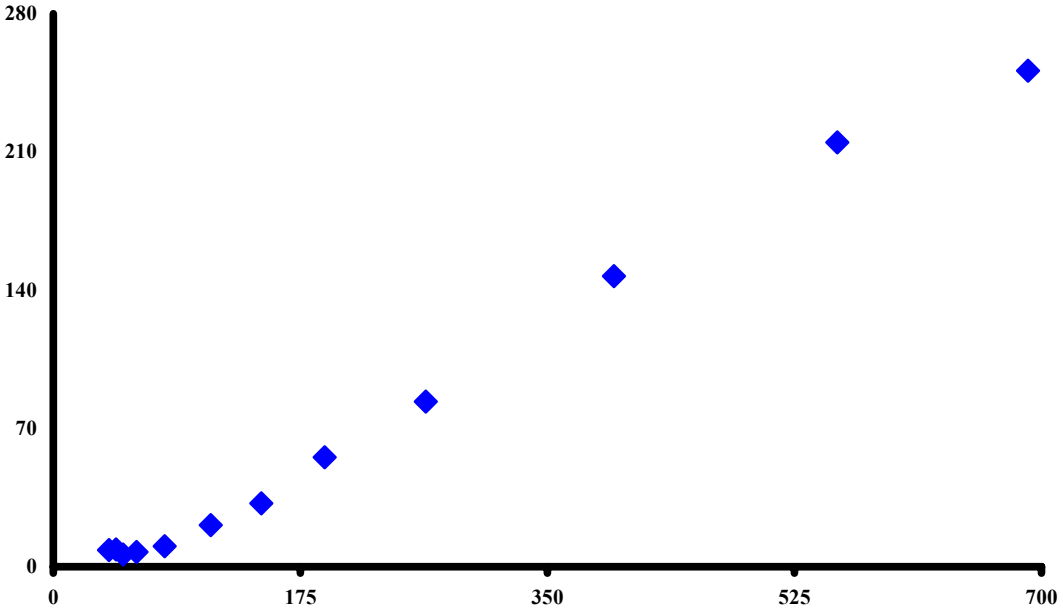
Zn-L & Cu-L Fluxes (nmol m^{-2} d^{-1})					
Season	**Sampling Date**	**Station 1**		**Station 2**	
		Zn-L	**Cu-L**	**Zn-L**	**Cu-L**
Fall	**October 29, 2001**	0	0	0	29
	(n = 3)	1900	0	0	-14
		2700	0	0	0
Summer	**June 26, 2001**	0	0	0	1522
	(n = 2-3)	0	376	0	675
			0		337
	June 28, 2002	0	997	2000	516
	(n = 3)	0	227	0	219
		0	1372	0	0
Spring	**April 23, 2001**	0	0	0	0
	(n = 2-3)	-440	0	0	376
			0		0

Table 8. Zn-ligand and Cu-ligand fluxes for individual core measurements at Station 1 and Station 2 for each sampling date. Negative values indicate a flux into the sediment. Positive values indicate flux out of the sediment. Non-statistically significant fluxes are represented as "0" net flux. n = number of cores per sampling site.

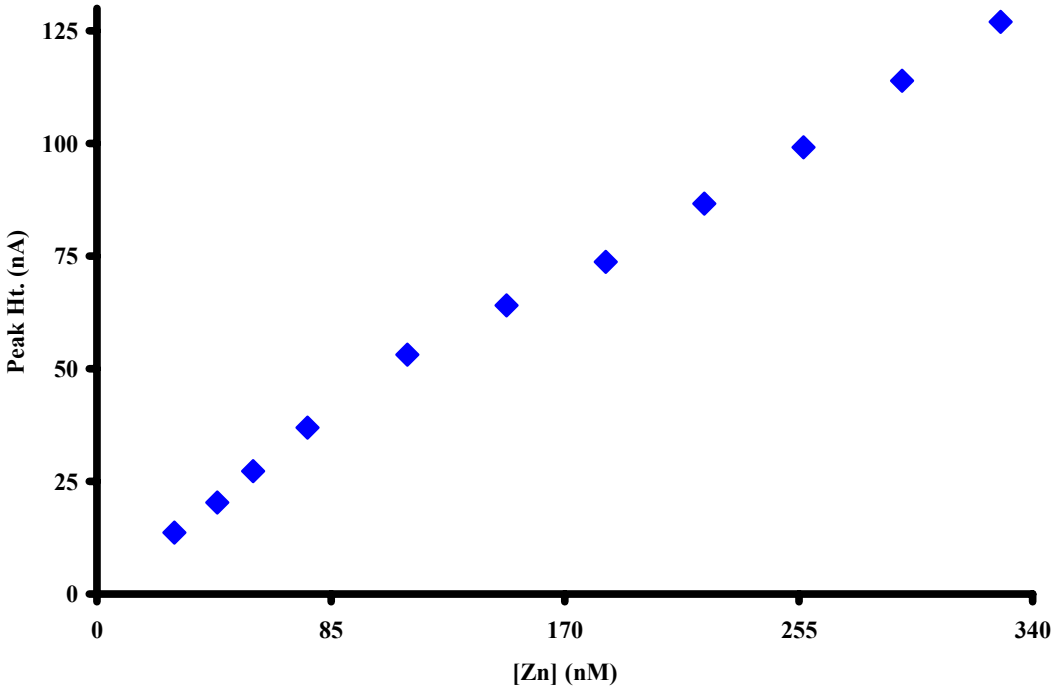
flux of Cu-L. Likewise, during June 2001 (Station 2) and June 2002 (Station 1), significant Cu-L fluxes occurred but Zn-L fluxes were not significant. These observations are consistent with water column measurements showing that complexation of Zn and Cu are not connected. The sediment-water exchange of Zn and Zn-complexing ligands appears to be decoupled from that of Cu and Cu-complexing ligands in the CFE, indicating that the ligands that complex the two metals are different.

Characterization of Zn Ligands

Samples containing Zn-complexing ligands typically had a titration curve (peak height vs. [*Zn*]) containing two distinguishable regions: an initially suppressed response followed by a linear response (Figure 10a). The suppressed response was caused by complexation of added Zn by excess natural organic ligands. When the excess ligands were completely titrated the current response became linear with respect to added zinc. Titration curves were linearized by a plot of $[Zn^{2+}]/[Zn\text{-}L]$ vs. $[Zn^{2+}]$. All Ruzic-van den Berg linearizations generally yielded straight lines characteristic of a one-ligand system (Figure 11). Scatchard plots of $[Zn\text{-}L]/[Zn']$ vs. $[Zn\text{-}L]$ for each titration curve also gave a single straight line, indicating a single ligand class. The conditional stability constants, K^{cond}, of the Zn-ligand complexes ranged from $10^{7.0}$ to $10^{9.2}$, which is consistent with other studies where K^{cond} ranged from $10^{7.4}$ to $10^{9.4}$ (van den Berg et al., 1986, 1987; Muller and Kester, 1991; Gardner, 1999). Occasionally the Ruzic-van den Berg plot was too scattered for linearization, usually when Zn-ligand concentrations were near the detection limit. In order to obtain a Zn-ligand concentration in these cases, the linear portion of the titration curve was extrapolated back to the x-axis. In order to verify the extrapolations were valid, samples were compared by both Ruzic-van den Berg



a.



b.

Figure 10. Typical titration curve in the (a) presence and (b) absence of Zn-complexing ligands. (a) October 2001, Station 1, Timepoint T5. (b) June 2002, Station 2, Timepoint T5.

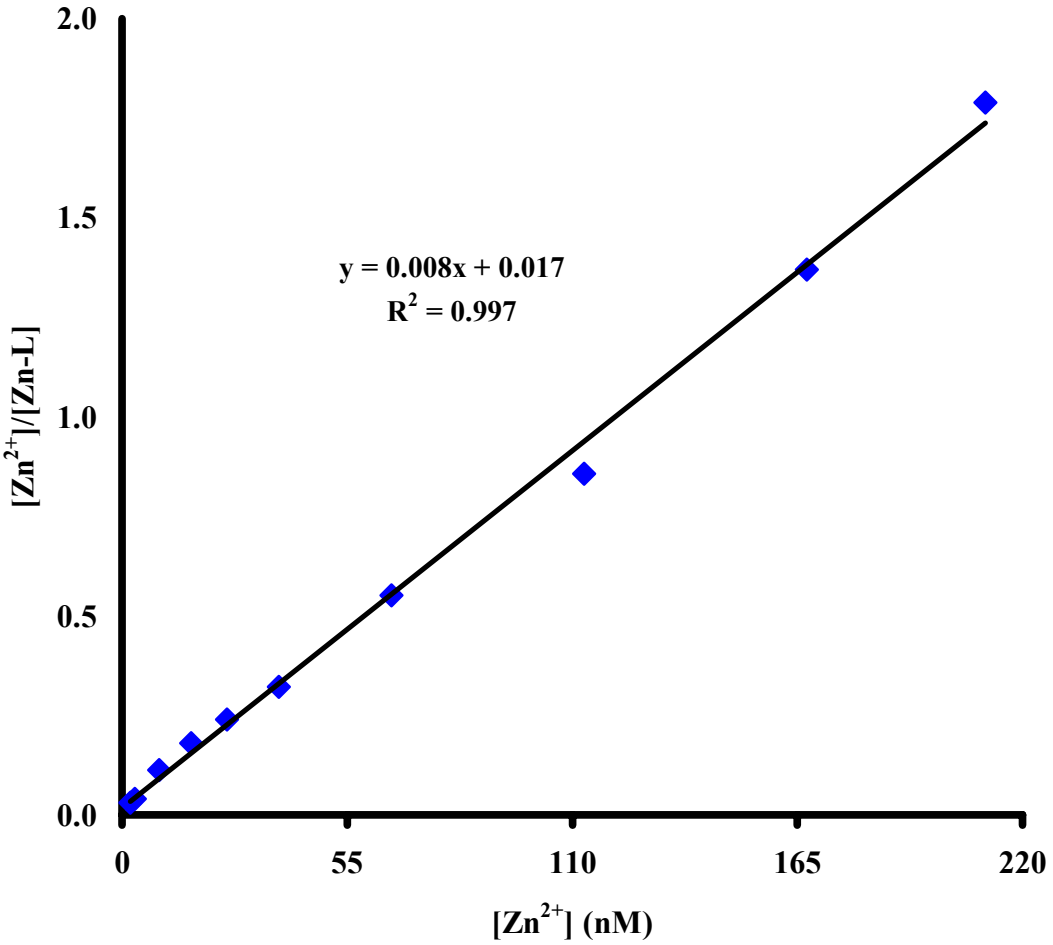


Figure 11. Typical Ruzic-van den Berg linearization of a titration curve indicating the presence of one Zn-complexing organic ligand. Cape Fear estuary, March 2002, Station 2, Timepoint T0.

linearization and extrapolation. Both techniques gave similar Zn-ligand concentrations. When the sample contained a very small concentration of ligands or ligands that were too weak to compete with APDC, then titration curves only gave a linear response to added zinc (Figure 10b). Zn-ligands were then reported as non-detectable. This response can be seen in Station 2 samples during April 2001 and June 2002. These observations of such small ligand concentrations was somewhat surprising given the organic-rich nature of the CFE and of the previous observations by Shank (2003) of the large excess of strong Cu-complexing ligands in the estuary. However, these results are consistent with the assertion that Zn and Cu complexation are decoupled in the sense that different ligands must be responsible for controlling the speciation of each metal.

CONCLUSIONS

Dissolved zinc speciation was determined seasonally in the water column and in benthic flux experiments at two contrasting sites in the Cape Fear estuary (CFE). Concentrations of TDZn and Zn-L in the Cape Fear estuary water column were relatively small compared to other impacted estuaries.

Previous studies of Cu speciation and sediment-water exchange in the Cape Fear estuary provided a comparison between Zn and Cu. Concentrations of Zn-L were correlated to TDZn concentrations only in the lower estuary, but not in the estuary overall. Organically complexed Zn ranged widely from 25 to 84%. In contrast, previous work showed that Cu-complexing ligand concentrations were always much greater than total dissolved Cu concentrations, and >99.9 % of Cu was organically complexed throughout the estuary. Zn-L did not correlate with DOC or river flow in the CFE, whereas Cu was highly correlated with DOC and river flow. Humic substances extracted

from Cape Fear freshwater did not detectably complex dissolved Zn. In contrast, previous studies have shown that humic substances from the CFE are very strong Cu complexants. Therefore, the ligands complexed to these metals are different and processes affecting the organic complexation of Cu and Zn appear to be decoupled in the Cape Fear estuary.

Statistically significant fluxes of TDZn were only observed in 40% of incubated cores of bottom sediments at both sampling stations over all sampling periods. Only 27% of the cores showed statistically significant fluxes of Zn-complexing ligands. The sporadic nature of benthic fluxes of both TDZn and Zn-complexing ligands suggests that sediments are an insignificant source, or at least not a continual source, of both constituents to overlying waters of the CFE. However, the ligands that flux out of the sediment are indistinguishable from those in the water column suggesting that at least a portion of the water column ligands could be derived from sedimentary processes. Given that the data do not show a strong river-dominated source (i.e. humics) for Zn-complexing ligands, sporadic sources within estuaries (such as benthic fluxes) may play an important role in overall Zn-ligand cycling.

Previous studies of Cu speciation in the CFE show that significant benthic fluxes of Cu-complexing ligands did not coincide with fluxes of significant Zn-complexing ligand fluxes further proving ligands complexed to these metals are different and their behaviors are decoupled in the Cape Fear estuary.